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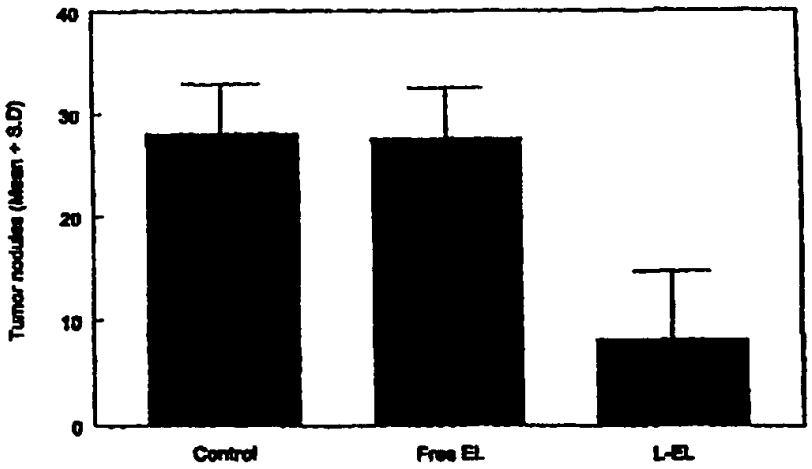

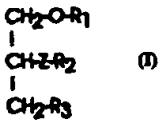

(I)

(57) Abstract

Ether lipids having formula (I), are incorporated into liposomes with a headgroup-derivatized lipid, and optionally a sterol and a neutral lipid. The liposomes can be used to administer ether lipids to animals such as humans, for example, for the treatment of cancers and inflammatory diseases and disorders.

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ETHER LIPID LIPOSOMES AND THEIR THERAPEUTIC USE

This invention is directed to ether lipid liposomes, and to their therapeutic use, for example, in the treatment of cancers.

Ether lipids are synthetic analogues of platelet activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine), an effector generally believed to be involved in a variety of physiological processes, such as inflammation, the immune response, allergic reactions and reproduction. Ether lipids have been shown to be effective antitumor agents in animals, and are believed to be selectively cytotoxic to a broad variety of cancer cells (see, for example, Dietzfelbinger et al. (1993); Zeisig et al. (1993); Powis et al. (1990); Berdel (1991); Bhatia and Hadju (1991); Reed et al. (1991); Workman (1991); Workman et al. (1991); Bazill and Dexter (1990); Berdel (1990); Counsell et al. (1990); Tritton and Hickman (1990); Muschiol et al. (1990); Layton et al. (1980); Runge et al. (1980); Great Britain Patent No. 1,583,661; U.S. Patent No. 3,752,886). Ether lipids have also been shown to be antimetastatic and anti-invasive, and to be capable of cell differentiation induction. None of these disclosures teach liposomal ether lipids with the advantageous properties of this invention's liposomal formulations. Several ether lipids are currently the subject of clinical trial study.

Mechanisms of ether lipid cytotoxicity, while not definitively established, appear to involve action at, and possible disruption of, the cell membrane. The selective cytotoxicity of ether lipids may involve intracellular accumulation and differential activity of alkyl cleavage enzymes. Ether lipids may also be selective inhibitors of phosphatidylinositol phospholipase C and protein kinase C activities, as well as of phosphatidylcholine biosynthesis.

Ether lipids can, in addition to their antitumor activity, be hemolytic, that is they can lyse red blood cells. Furthermore, clinical

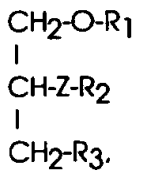
trial results show that ether lipid administration can lead to hepatic dysfunction and gastrointestinal disorders. Applicants have found that certain liposomal formulations of ether lipids can buffer these toxicities.

SUMMARY OF THE INVENTION

A liposome having a lipid bilayer comprising a headgroup-derivatized lipid and an ether lipid is provided herein. Preferably, the liposome has a diameter of less than about 200 nm, more preferably, from greater than about 50 nm to less than about 200 nm.

The headgroup-derivatized lipid is preferably a phosphatidylethanolamine-dicarboxylic acid. The dicarboxylic acid, for example, glutaric, sebacic, succinic, or tartaric acid, is preferably glutaric acid. The phosphatidylethanolamine (PE) is preferably dipalmitoyl-phosphatidylethanolamine (DPPE), palmitoyloleoyl phosphatidylethanolamine (POPE) or dioleoyl phosphatidylethanolamine (DOPE). More preferably, the headgroup-derivatized lipid is DOPE-GA. The headgroup-derivatized lipid can, but is not required to, be a circulation-enhancing lipid.

The ether lipid has the chemical formula:



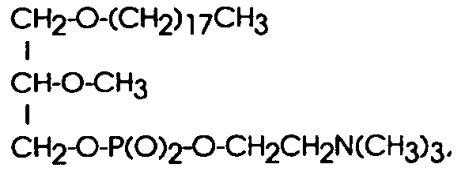
wherein R_1 is Y_1Y_2, Y_2 is CH_3 or CO_2H and Y_1 is $(CH_2)_{n1}(CH=CH)_{n2}(CH_2)_{n3}(CH=CH)_{n4}(CH_2)_{n5}(CH=CH)_{n6}(CH_2)_{n7}(CH=CH)_{n8}(CH_2)_{n9}$. n1 is equal to zero or an integer of from 3 to 23; n3 is equal to zero or an integer of from 1 to 21; n5 is equal to zero or an integer of from 1 to 18; n7 is equal to zero or an integer of from 1 to 15; n9 is equal to zero or an integer of from 1 to 12; and each of n2, n4, n6 and 8 is independently equal to zero or 1. The sum of n1 +2n2 +n3 +2n4 +n5 +2n6 +

n7 +2n8 +n9 is equal to an integer of from 3 to 23. Y_2 is preferably CH_3, and R_1 is preferably $(CH_2)_{n1}CH_3$, that is, a saturated hydrocarbon. The preferred saturated hydrocarbon is R_1 is $(CH_2)_{17}CH_3$.

Z is preferably O, but can also be S. R_2 can also be Y_1Y_2, and can be the same as, or different than, R_1. Preferably, R_2 is $(C(X_1)_{n10}(X_2)_{n11})_{n12}CX_3X_4X_5$. Each of X_1, X_2, X_3, X_4, and X_5 is independently hydrogen or fluorine, but is preferably hydrogen. n10 is equal to zero, 1 or 2; n11 is equal to zero, 1, or 2; and n12 is equal to zero or an integer of from 1 to 23, but is preferably, zero. When n12 is zero, R_2 is $CX_3X_4X_5$. X_3, X_4, and X_5 are preferably H, and R_2 is CH_3. When n12 is not zero, the sum of n10 + n11 is equal to 2, n12 is preferably equal to 1, and R_2 is preferably CH_2CH_3, CH_2CF_3 or CF_2CF_3.

R_3 has the formula R_5-P(O)$_2$-O-R_6, R_5 is O, S or NH, but is preferably O, and R_6 is $CH_2CH_2N(CH_3)_3$ (choline) $CH_2CH_2NH_2$, $CH_2CH(OH)CH_2OH$, or $CH_2CH_2NHC(O)R_7$, but is preferably choline. Accordingly, R_3 is preferably -O-P(O)$_2$-O-$CH_2CH_2N(CH_3)_3$. R_7 is Y_2CH_3 or Y_2CO_2H.

Accordingly, the preferred ether lipid is:



that is, the ether lipid is 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine ("ET-18-OCH_3").

The liposome of this invention can also comprise a neutral lipid, such as a phosphatidylcholine (PC). Preferably, the PC is egg phosphatidylcholine (EPC), dioleoyl phosphatidylcholine (DOPC) or palmitoyloleoyl phosphatidylcholine (POPC). More preferably, the PC is DOPC. The liposome can also comprise a sterol, preferably, cholesterol. The liposome can also comprise a circulation-enhancing lipid, which can

comprise a phosphatidylethanolamine, and a dicarboxylic acid, ganglioside or polyethylene glycol.

In a preferred embodiment of the invention, the liposome has a lipid bilayer comprising an ether lipid, a headgroup-derivatized lipid, a sterol and a neutral lipid. Preferably, the ether lipid is ET-18-OCH_3, the headgroup-derivatized lipid is a phosphatidylethanolamine-dicarboxylic acid, more preferably, DOPE-GA, the sterol is cholesterol and the neutral lipid is a PC, more preferably, DOPC. The bilayer generally comprises from about 10 mole percent to about 30 mole percent, more preferably about 20 mole percent, of the ether lipid, from about 5 mole percent to about 20 mole percent, more preferably about 10 mole percent of the headgroup-derivatized lipid, from about 10 mole percent to about 50 mole percent, more preferably about 30 mole percent of the sterol and from about 10 mole percent to about 50 mole percent, more preferably about 40 mole percent of the neutral lipid. Most preferably, the bilayer comprises about 20 mole percent "ET-18-OCH_3", about 10 mole percent DOPE-GA, about 30 mole percent cholesterol and about 40 mole percent DOPC.

The liposome can comprise an additional bioactive agent, which is preferably an antimicrobial agent, antineoplastic agent, anti-inflammatory agent, therapeutic lipid or hematopoietic cell growth stimulating agent. The liposome can be dehydrated.

Also provided herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and the ether lipid liposome of this invention. Further provided is a method of administering an ether lipid to an animal which comprises administering to the animal a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a liposome having a lipid bilayer which comprises an ether lipid and a headgroup-derivatized lipid, the ether lipid having the chemical formula:

$$\begin{array}{l} CH_2\text{-}O\text{-}R_1 \\ | \\ CH\text{-}Z\text{-}R_2 \\ | \\ CH_2\text{-}R_3, \end{array}$$

Preferably the animal is a human. Preferably, the liposome has a diameter of less than about 200 nm, more preferably, from greater than about 50 nm to less than about 200 nm.

Preferably, a saturated hydrocarbon, most preferably, $(CH_2)_{17}CH_3$ is attached at the 1 position of the ether lipid through the O. Preferably, R_2 is $(C(X_1)_{n10}(X_2)_{n11})_{n12}CX_3X_4X_5$, more preferably, CH_3. Preferably, the polar group at the 3 position is phosphorylcholine. Accordingly, the ether lipid is preferably 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine (ET-18-OCH_3).

Preferably, the headgroup-derivatized lipid is a PE-dicarboxylic acid,, most preferably, DOPE-GA. The derivatized lipid can be a circulation-enhancing lipid. Preferably the bilayer comprises cholesterol and a phosphatidylcholine, more preferably, EPC, POPC or DOPC. The liposome can also comprise a circulation-enhancing lipid

In a particularly preferred embodiment of the invention, the liposome comprises 20 mole percent of an ether lipid, preferably ET-18-OCH_3, about 10 mole percent DOPE-GA, about 30 mole percent cholesterol and about 40 mole percent DOPC.

The method of this invention can be used to administer an ether lipid to an animal afflicted with a cancer, including, but not limited to, carcinomas, leukemias, myelomas, neuroblastomas, sarcomas, of the lungs, brain, ovaries, colon or breasts. Such a method generally comprises administering to the animal an amount of the liposome which comprises an antineoplastic effective amount of the ether lipid. Antineoplastic effective amounts of the liposomal ether lipid are generally from about 0.1 mg of the ether lipid per kg of the body weight of the animal treated to about 1000 mg of the ether lipid per kg of body weight. Preferably, the antineoplastic effective amount of the liposomal ether lipid is from about 1 mg of the ether lipid per kg of body weight to about 500 mg per kg. Most preferably, the anticancer effective amount of the ether lipid is 200 mg per kg of body weight. Anticancer effective amounts of a liposomal ether

lipid can be administered in multiple administrations. Multiple doses of a liposomal ether lipid, each dose comprising an anticancer effective amount of the ether lipid, can also be administered. Metastases or invasion of the cancer can be inhibited by ether lipid administration.

The method of this invention can also be used to administer an ether lipid to an animal afflicted with an inflammatory disorder, e.g., an arthritic condition, allergic reaction or asthmatic disorder. Such a method generally comprises administering an anti-inflammation effective amount of the liposomal ether lipid to the animal. Anti-inflammation effective amounts of ether lipids are generally from about 0.1 mg of the ether lipid per kg of the body weight of the animal treated to about 1000 mg of the ether lipid per kg of body weight.

The method of this invention can also comprise administration of a biologically active agent in addition to the ether lipid administered. This additional biologically active agent, which can be associated, or unassociated with the liposome, is, for example, but not limited to, an antimicrobial agent, antineoplastic agent, anti-inflammatory agent, therapeutic lipid or hematopoietic cell growth stimulating agent.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. Effects of Liposomal Ether Lipid on Lung Metastasis in Mice. Liposomal formulation: EPC/Cholesterol/DPPE-GA/EL (4:3:1:2). Dose: 25 mg/kg x 5. x-axis: Buffer control, liposome control (no ether lipid), free ether lipid, liposomal ether lipid; y-axis: number of tumor nodules (mean plus s.d.).

FIGURE 2 Effects of Liposomal Ether Lipid on Lung Metastasis in Mice. Liposome formulation: DOPC/Chol/DOPE-GA/EL (4:3:1:2). Dose: 5 injections. x-axis: Buffer control, liposome control, liposome control, free ether lipid,

liposomal ether lipid (25 mg/kg of body weight dose), liposomal ether lipid (100 mg per kg dose); y-axis: number of tumor nodules (mean plus s. d.

DETAILED DESCRIPTION OF THE INVENTION

Provided herein is a liposome having a lipid bilayer comprising a headgroup-derivatized lipid and an ether lipid. Liposomes are self-assembling structures comprising one or more lipid bilayers, each of which surrounds an aqueous compartment and comprises two opposing monolayers of amphipathic lipid molecules. These comprise a polar (hydrophilic) headgroup region covalently linked to one or two non-polar (hydrophobic) acyl chains. Energetically unfavorable contacts between the hydrophobic acyl chains and the aqueous medium are generally believed to induce lipid molecules to rearrange such that the polar headgroups are oriented towards the aqueous medium while the acyl chains reorient towards the interior of the bilayer. An energetically stable structure is formed in which the acyl chains are effectively shielded from coming into contact with the aqueous medium.

Liposomes can be made by a variety of methods (for a review, see, for example, Deamer and Uster (1983)). These methods include without limitation: Bangham's methods for making muiltilamellar liposomes (MLVs); Lenk's, Fountain's and Cullis' methods for making MLVs with substantially equal interlamellar solute distribution (see, for example, U.S. Patent Nos. 4,522,803, 4,588,578, 5,030,453, 5,169,637 and 4,975,282); and Paphadjopoulos et al.'s reverse-phase evaporation method (U.S. Patent No. 4,235,871) for preparing oligolamellar liposomes. Unilamellar vesicles can be produced from MLVs by such methods as sonication (see Paphadjopoulos et al. (1968)) or extrusion (U.S. Patent No. 5,008,050 and U.S. Patent No. 5,059,421). The ether lipid liposome of this invention can be produced by the methods of any of these disclosures, the contents of which are incorporated herein by reference.

Various methodologies, such as sonication, homogenization, French Press application, milling and extrusion can be used to size reduce

liposomes, that is to prepare liposomes of a smaller size from larger liposomes. Tangential flow filtration (see WO89/008846), can also be used to regularize the size of liposomes, that is, to produce liposomes having a population of liposomes having less size heterogeneity, and a more homogeneous, defined size distribution. The liposome of this invention can be unilamellar or multilamellar, and preferably has a diameter of less than about 200 nm, more preferably, from greater than about 50 nm to less than about 200 nm.

A "headgroup-derivatized" lipid is a lipid which, when present in a liposomal lipid bilayer with an ether lipid, can buffer the toxicity of the ether lipid, that is, can decrease the ether lipid's toxicity, such that it is generally less toxic than the free form of the ether lipid. Headgroup-derivatized lipids generally are amphipathic lipids comprising one or more hydrophobic acyl chains, and a polar headgroup to which a chemical moiety has been attached. The acyl chains typically contain from 4 to 24 carbon atoms, and can be saturated or unsaturated. Preferred acyl chains are those which can adopt compatible packing configurations with the hydrophobic portions of other lipids present in the bilayer, and which can interact with an ether lipid such that release of the ether lipid from the bilayer is inhibited and ether lipid toxicity is buffered. Preferred polar groups are those, such as ethanolamine, to which chemical moieties can be attached. Suitable chemical moieties are those, such as dicarboxylic acids, gangliosides, polyethylene glycols, polyakyl ethers and the like, preferably dicarboxylic acids, which can be attached to polar groups and which give rise to headgroup-derivatized lipids. Means of identifying chemical moieties suitable for attachment to groups such as ethanolamine, for example by subjecting derivatized lipids to in vitro and in vivo toxicity testing, are well known to, and readily practiced by, ordinarily skilled artisans given the teachings of this invention. Means of attaching chemical moieties to polar groups are also well known to, and readily practiced by, ordinarily skilled artisans.

Preferred acyl chains, which include palmitate and oleate chains, have from 16 to 20 carbon atoms. More preferably, one or more of the acyl chains, such as an oleate chain, is unsaturated. The preferred polar

group is ethanolamine. Moities preferred for attachment of ethanolamine are dicarboxylic acids, including those which are saturated, or unsaturated, and which have from 4 to 24 carbon atoms, such as glutaric, sebacic, succinic and tartaric acid. More preferably, the dicarboxylic acid is glutaric acid. Accordingly, preferred headgroup-derivatized lipids are phosphatidylethanolamine-dicarboxylic acids such as dipalmitoyl phosphatidylethanolamine-glutaric acid (DPPE-GA), palmitoyloleoyl phosphatidylethanolamine-glutaric acid ((POPE-GA) and dioleoyl phosphatidylethanolamine-glutaric acid (DOPE-GA). Most preferably, the derivatized lipid is DOPE-GA. Typically, the headgroup-derivatized lipid comprises from about 5 mole percent to about 50 mole percent of the liposome's lipid bilayer.

Toxicity buffering capacities of headgroup-derivatized lipids can be determined by a number of in vitro and in vivo testing methods well known to, and readily practiced by, ordinarily skilled artisans, given the teachings of this invention. For example, ether lipid-induced red blood cell (RBC) hemolysis can be examined in vitro by combining an ether lipid with an RBC suspension, incubating the combination, and then quantitating the percentage of RBC lysis by spectrophotometry. The data presented below show that the concentration of free ether lipid inducing hemolysis of five percent of an RBC suspension (HI_5) was 5.2 micromolar, while the HI_5 values for ether lipids in liposomes also having a headgroup-derivatized lipid ranged from 8.9 micromolar to 106 micromolar.

Additionally, LD_{50}'s for ether lipids can be determined in vivo by injecting a range of ether lipid doses into sample groups of suitable test animals, e.g., mice; the number of animals expiring in each group is determined, and the ether lipid dose at which fifty percent of the animals in expire is determined. The data presented below show that at liposomal ether lipid doses of 100 and 130 mg of ether lipid/kg body weight, none of the treated animals expired, while at a 100 mg/kg dose of free ether lipid, five of five mice in the tested group expired. The LD_{50} for free ether lipid (ET-18-OCH_3) was about 32.2 mg of the ether lipid per kg of body weight, while the LD_{50} for liposomal ether lipid was greater than 130 mg per kg.

Therapeutic window "TW" equals HI_5/GI_{50} ("GI_{50}" equals the dose of an agent inducing fifty percent growth inhibition in a population of cells exposed to the agent). Generally, when a bioactive agent's TW is less than 1, the agent cannot be administered to achieve 50% growth inhibition without causing generally unacceptable levels of hemolysis. Generally, when the TW is about 1 or greater, the bioactive agent can be administered without inducing generally unacceptable levels of hemolysis. Typically, the higher the TW value, the more therapeutically effective is the agent tested. Ether lipid liposomes having bilayers comprising headgroup-derivatized lipids can have TW's of greater than 1. Preferably, the TW of an ether lipid in a liposomal bilayer also comprising a headgroup-derivatized lipid is greater than about 1.5, more preferably, greater than about 2 and still more preferably, greater than about 3.

The following table presents TW's for free and liposomal ether lipids (1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine) in connection with A549 and MCF7 human cancer cells.

	TW	
Formulation	A549	MCF7
Free EL	0.78	0.26
DOPC:Chol:DOPE-GA:EL	7.36	3.61
EPC:Chol:POPE-GA:EL	2.77	0.96
Chol:DPPG:EL	2.14	>1.04

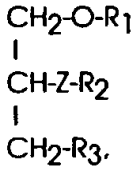
Data presented in the Examples below show that the hemolytic activity of EL decreased upon liposomal entrapment with a headgroup-derivatized lipid. The ether lipid concentrations inducing 50% red blood cell hemolysis (HI_{50}) were: free EL, 13 μM; EL-Lip-1; 43 μM;

EL-Lip-2, >180 μM. Ether lipid concentrations inducing 50% growth inhibition of A549 cancer cells (GI_{50}) were similar: free EL, 10.2 μM; EL-Lip-1, 12.4 μM; EL-Lip-2, 14.1 μM. Furthermore, the anti-metastatic activity of free EL and EL-Lip-1 against Lewis lung cancer in mice was compared. EL-Lip-1 was found to inhibit spontaneous lung metastasis, whereas free EL had only limited effectiveness (Control: 28 ± 4.9 lung nodules; free EL: 27.5 ± 5; EL-Lip-1: 8.1 ± 6.5).

Headgroup-derivatized lipids can also be circulation-enhancing lipids, that is, the modifications directed to lipid toxicity buffering can also be directed to circulation enhancement; derivatized lipids can inhibit clearance of liposomes from the circulatory systems of animals to which they have been administered. Liposomes are generally believed to be cleared from an animal's body by way of its reticuloendothelial system (RES). Avoiding RES clearance means that the frequency of liposome administration can be reduced, and that less of a liposome-associated bioactive agent need be administered to achieve desired serum levels of the a. Enhanced circulation times can also allow targeting of liposomes to non-RES containing tissues. Liposome outer surfaces are believed to become coated with serum proteins, such as opsonins, in animals' circulatory systems.

Without intending in any way to be limited by theory, it is believed that liposome clearance can be inhibited by modifying the outer surface of liposomes such that binding of serum proteins thereto is generally inhibited. Effective surface modification, that is, alterations to the outer surfaces of liposomes which result in inhibition of opsonization and RES uptake, is believed to be accomplished by incorporating into liposomal bilayers lipids whose polar headgroups have been derivatized by attachment thereto of a chemical moiety which can inhibit the binding of serum proteins to liposomes such that the pharmacokinetic behavior of the liposomes in the circulatory systems of animals is altered (see, e.g., Blume et al. (1993); Gabizon et al. (1993); Park et al. (1992); Woodle et al. U.S. Patent No. 5,013,556; U.S. Patent No. 4,837,028; and U.S. Patent Appln. No. 08/065,928, filed May 21, 1993).

The ether lipid has the chemical formula:



wherein R_1 is Y_1Y_2. Y_2 is CH_3 or CO_2H and Y_1 is $(CH_2)_{n1}(CH=CH)_{n2}(CH_2)_{n3}(CH=CH)_{n4}(CH_2)_{n5}(CH=CH)_{n6}(CH_2)_{n7}(CH=CH)_{n8}(CH_2)_{n9}$. n1 is equal to zero or an integer of from 3 to 23; n3 is equal to zero or an integer of from 1 to 21; n5 is equal to zero or an integer of from 1 to 18; n7 is equal to zero or an integer of from 1 to 15; n9 is equal to zero or an integer of from 1 to 12; and each of n2, n4, n6 and 8 is independently equal to zero or 1. The sum of n1 +2n2 +n3 +2n4 +n5 +2n6 + n7 +2n8 +n9 is equal to an integer of from 3 to 23. Accordingly, a hydrocarbon is attached, through an O atom at the 1 position, to the glycerol backbone of the ether lipid. The hydrocarbon can have a a terminal CH_3 or CO_2H group; preferably, the terminal group is a methyl group. The hydrocarbon is preferably saturated, that is, it preferably has no double bonds between adjacent carbon atoms, each of n2, n4, n6 and n8 are equal to zero, and R_1 is $(CH_2)_{n1}CH_3$. More preferably, R_1 is $(CH_2)_{17}CH_3$.

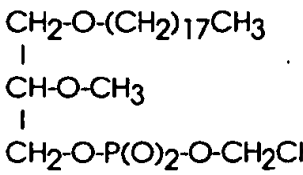
Alternatively, the hydrocarbon can have one or more double bonds, that is, it can be unsaturated, and one or more of n2, n4, n6 and n8 can be equal to 1. When the unsaturated hydrocarbon has one double bond, n2 is equal to 1, n4, n6 and n8 are each equal to zero and Y_1 is $(CH_2)_{n1}CH=CH(CH_2)_{n3}$. n1 is zero or an integer of from 1 to 21, and n3 is also zero or an integer of from 1 to 21, at least one of n1 or n3 not being equal to zero. When the hydrocarbon has two double bonds, n2 and n4 are each equal to 1, n6 and n8 are each equal to zero, and Y_1 is $(CH_2)_{n1}CH=CH(CH_2)_{n3}CH=CH(CH_2)_{n5}$. n1 and n5 are each independently zero or an integer of from 1 to 18, and n3 is an integer of from 1 to 16. When the hydrocarbon has three double bonds, n2, n4 and n6 are each equal to 1, n8 is equal to zero, and Y_1 is

$(CH_2)_{n1}CH=CH(CH_2)_{n3}CH=CH(CH_2)_{n5}CH=CH\ (CH_2)_{n7}$. n1 and n7 are each independently zero or an integer of from 1 to 15, and n3 and n5 are each equal to an integer of from 1 to 15. When the unsaturated hydrocarbon has four double bonds, each of n2, n4, n6 and n8 is equal to 1, and Y_1 is $(CH_2)_{n1}CH=CH(CH_2)_{n3}CH=CH(CH_2)_{n5}CH=CH(CH_2)_{n7}$ $CH=CH(CH_2)_{n9}$. n1 and n9 are each independently zero or an integer of from 1 to 12; n3, n5 and n7 are each independently an integer of from 1 to 12.

Z is preferably O, but can also be S. R_2 can also be Y_1Y_2, and can be the same or different than R_1. Preferably, R_2 is $(C(X_1)_{n10}(X_2)_{n11})_{n12}CX_3X_4X_5$. Each of X_1, X_2, X_3, X_4, and X_5 is independently H or F, but is preferably H, n10 is equal to zero, 1 or 2, n11 is equal to zero, 1, or 2; and n12 is equal to zero or an integer of from 1 to 23. Preferably, n12 is equal to zero, in which case, R_2 is $CX_3X_4X_5$. X_3, X_4 and X_5 are preferably H, and R_2 is preferably CH_3. When n12 is not zero, the sum of n10 + n11 is 2, n12 is preferably equal to 1, and R_2 can be CH_2CH_3, CH_2CF_3 or CF_2CF_3.

R_3 is R_5-$P(O)_2$-O-R_6, R_5 being O, S or NH, but preferably O. R_6 can be $CH_2CH_2N(CH_3)_3$ (phosphorylcholine) $CH_2CH_2NH_2$ (phosphorylserine), $CH_2CH(OH)CH_2OH$ (phosphorylglycerol), or $CH_2CH_2NHC(O)R_7$, but is preferably phosphorylcholine. R_3 is therefore preferably, but not necessarily, -O-$P(O)_2$-O-$CH_2CH_2N(CH_3)_3$. R_7 is Y_2CH_3 or Y_2CO_2H.

Accordingly, the preferred ether lipid is:



that is, 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine ("ET-18-OCH_3").

The liposome can comprise additional lipids, that is, one or more lipids in addition to the headgroup-derivatized lipid and ether lipid

Additional lipids are selected for their ability to adapt compatible acyl chain packing conformations with the other components of the lipid bilayer such that the lipid constituents are tightly packed and release of the lipids from the bilayer is inhibited. Lipid-based factors contributing to compatible packing conformations are well known to ordinarily skilled artisans. Such factors include, without limitation, the acyl chain length and degree of unsaturation, as well as the headgroup size and charge. Accordingly, suitable additional lipids can readily be selected by ordinarily skilled artisans given the teachings of this invention.

Preferred additional lipids include neutral lipids such as the phosphatidylcholines (PC) egg phosphatidylcholine (EPC), palmitoyloleoyl phosphatidylcholine (POPC) and dioleoyl phosphatidylcholine (DOPC). Preferred PC's have one or two acyl chains, such as DOPC which has two unsaturated chains. The bilayer of this invention's liposome can also comprise a sterol. Sterols generally affect the fluidity of lipid bilayers (see, for example, Lewis and McElhaney (1992) and Darnell et al. (1986)) Accordingly, sterol interactions with surrounding hydrocarbon chains generally inhibit emigration of these chains from the bilayer. The sterol component of this invention's bilayer is preferably, but not necessarily, cholesterol. The sterol can also be cholesterol sulfate or cholesterol hemisuccinate. The liposome can also comprise a circulation-enhancing lipid, that is, such a lipid in addition to the headgroup-derivatized lipid, which can, but is not necessarily, a circulation-enhancing lipid.

Preferably, the liposome of this invention has a bilayer comprising an ether lipid, a headgroup-derivatized lipid, a sterol and a neutral lipid. Preferably, the ether lipid is ET-18-OCH_3, the headgroup-derivatized lipid is a PE-dicarboxylic acid, the sterol is cholesterol and the neutral lipid is a PC. Most preferably, the PE-dicarboxylic acid is DOPE-GA, and the PC is DOPC. Typically, the bilayer comprises from about 10 to about 30 mole percent ether lipid, from about 5 to about 20 mole percent of the headgroup-derivatized lipid, from about 10 to about 50 mole percent of the sterol and from about 10 to about 50 mole percent of the neutral lipid. In a particularly preferred embodiment of this invention, the bilayer comprises

about 20 mole percent ET-18-OCH_3, about 10 mole percent DOPE-GA, about 30 mole percent cholesterol and about 40 mole percent DOPC.

The liposome can comprise an additional bioactive agent, that is, a bioactive agent in addition to the ether lipid. A "bioactive agent" is any compound or composition of matter that can be administered to animals, preferably humans. Such agents can have biological activity in animals; the agents can also be used diagnostically in the animals. Bioactive agents include therapeutic and imaging agents. Bioactive agents which may be associated with liposomes include, but are not limited to: antiviral agents such as acyclovir, zidovudine and the interferons; antibacterial agents such as aminoglycosides, cephalosporins and tetracyclines; antifungal agents such as polyene antibiotics, imidazoles and triazoles; antimetabolic agents such as folic acid, and purine and pyrimidine analogs; antineoplastic agents such as the anthracycline antibiotics and plant alkaloids; sterols such as cholesterol; carbohydrates, e.g., sugars and starches; amino acids, peptides, proteins such as cell receptor proteins, immunoglobulins, enzymes, hormones, neurotransmitters and glycoproteins; dyes; radiolabels such as radioisotopes and radioisotope-labelled compounds; radiopaque compounds; fluorescent compounds; mydriatic compounds; bronchodilators; local anesthetics; and the like. Liposomal bioactive agent formulations can enhance the therapeutic index of the bioactive agent, for example by buffering the agent's toxicity. Liposomes can also reduce the rate at which a bioactive agent is cleared from the circulation of animals. Accordingly, liposomal formulation of bioactive agents can mean that less of the agent need be administered to achieve the desired effect. Additional bioactive agents preferred for the liposome of this invention include antimicrobial, anti-inflammatory and antineoplastic agents, or therapeutic lipids, for example, ceramides. Most preferably, the additional bioactive agent is an antineoplastic agent.

Liposomes can be loaded with one or more biologically active agents by solubilizing the agent in the lipid or aqueous phase used to prepare the liposomes. Alternatively, ionizable bioactive agents can be loaded into liposomes by first forming the liposomes, establishing an

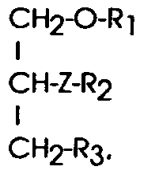
electrochemical potential, e.g., by way of a pH gradient, across the outermost liposomal bilayer, and then adding the ionizable agent to the aqueous medium external to the liposome (see Bally et al. U.S. Patent No. 5,077,056 and WO86/01102).

The liposome of this invention can be dehydrated, stored and then reconstituted such that a substantial portion of their internal contents are retained in the liposomes. Liposomal dehydration generally requires use of a hydrophilic drying protectant (see U.S. Patent Nos. 4,229,360 and 4,880,635). This hydrophilic compound is generally believed to prevent the rearrangement of the lipids in the liposome, so that the size and contents are maintained during the drying procedure and through rehydration, such that the liposomes can be reconstituted. Appropriate qualities for such drying protectants are that they be strong hydrogen bond acceptors, and possess stereochemical features that preserve the intramolecular spacing of the liposome bilayer components. Saccharide sugars, preferentially mono- and disaccharides, are suitable drying protectants for liposomes. Alternatively, the drying protectant can be omitted if the liposome preparation is not frozen prior to dehydration, and sufficient water remains in the preparation subsequent to dehydration.

Also provided herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and the liposome of this invention. "Pharmaceutically acceptable carriers" as used herein are generally intended for use in connection with the administration of lipids and liposomes, including liposomal bioactive agent formulations, to animals, including humans. Pharmaceutically acceptable carriers are generally formulated according to a number of factors well within the purview of the ordinarily skilled artisan to determine and account for, including without limitation: the particular liposomal bioactive agent used, its concentration, stability and intended bioavailability; the disease, disorder or condition being treated with the liposomal composition; the subject, its age, size and general condition; and the composition's intended route of administration, e.g., nasal, oral, ophthalmic, topical, transdermal, vaginal, subcutaneous, intramammary, intraperitoneal, intravenous, or intramuscular (see, for example, Nairn (1985)). Typical pharmaceutically acceptable carriers

used in parenteral bioactive agent administration include, for example, D5W, an aqueous solution containing 5% weight by volume of dextrose, and physiological saline. Pharmaceutically acceptable carriers can contain additional ingredients, for example those which enhance the stability of the active ingredients included, such as preservatives and anti-oxidants.

Further provided is a method of administering an ether lipid to an animal which comprises administering to the animal a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a liposome having a lipid bilayer which comprises a headgroup-derivatized lipid and the ether lipid. The ether lipid has the chemical formula:



Preferably the animal is a human, and the ether lipid is administered intravenously. Preferably, the liposome has a diameter of less than about 200 nm, more preferably, from greater than about 50 nm to less than about 200 nm.

The headgroup-derivatized lipid is preferably a phosphatidylethanolamine-dicarboxylic acid, the acid preferably being glutaric acid and the PE preferably being DPPE, POPE or DOPE. More preferably, the headgroup-derivatized lipid is DOPE-GA. The headgroup-modified lipid can also be a circulation-enhancing lipid.

Preferably, a saturated hydrocarbon, more preferably $CH_3(CH_2)_{17}$- is attached at the 1 position of the ether lipid through an O; preferably, a methyl group is attached at the 2 position through an O; and preferably, the polar group at the 3 position is phosphorylcholine. Accordingly, the preferred ether lipid is 1-O-octadecyl-2-O-methyl-3-sn-glycero-phosphocholine ("ET-18-OCH_3"). Preferably, the liposome's lipid bilayer also comprises a sterol, more preferably, cholesterol, and a neutral lipid, such

as a PC, and preferably DOPC. The bilayer can also comprise a circulation-enhancing lipid.

Preferably, the liposome used in the method of this invention has a lipid bilayer comprising ET-18-OCH_3, DOPE-GA, cholesterol and DOPC. More preferably, the bilayer comprises about 40 mole percent DOPC, about 30 mole percent cholesterol, about 10 mole percent of DOPE-GA and about 20 mole percent of the ether lipid.

Ether lipids are generally believed to be selectively cytotoxic to tumor cells. Accordingly, the method of this invention can be used to treat animals afflicted with a cancer, including, but not limited to, a carcinoma, leukemia, myeloma, neuroblastoma, or a sarcoma, of the lung, brain, ovarian, colon or breast. Generally, liposomal ether lipids can be used to treat cancers treated with free, that is, nonliposomal, ether lipids. However, encapsulation of an ether lipid in a liposome can enhance its therapeutic index, and therefore make the liposomal ether lipid a more effective treatment. The method is practiced by administering to the animal an antineoplastic effective amount of the liposomal ether lipid of this invention. Administration of the liposomal ether lipid can inhibit metastases of cancerous cells, or their invasion of tissues.

For the purposes of this invention, "anticancer effective amounts" of liposomal ether lipids are amounts effective to inhibit, ameliorate, lessen or prevent establishment, growth, metastasis or invasion of one or more cancers in animals to which the ether lipids have been administered. Anticancer effective amounts are generally chosen in accordance with a number of factors, e.g., the age, size and general condition of the subject, the cancer being treated and the intended route of administration, and determined by a variety of means, for example, dose ranging trials, well known to, and readily practiced by, ordinarily skilled artisans given the teachings of this invention. Antineoplastic effective amounts of the liposomal ether lipid of this invention are generally expected to be about the same as such amounts of free, nonliposomal, ether lipids, and range from about 0.1 mg of the ether lipid per kg of the body weight of the

animal treated to about 1000 mg of the ether lipid per kg of body weight. Preferably, the antineoplastic effective amount of the ether lipid is from about 1 mg of the ether lipid per kg of body weight to about 500 mg per kg. More preferably, the anticancer effective amount of the liposomal ether lipid is from about 5 mg/kg to about 100 mg/kg.

The method of this invention can also be used to administer an ether lipid to an animal afflicted with an anti-inflammatory disorder. Inflammatory disorders treatable by the method of this invention include, without limitation: arthritic conditions such as gout, rheumatoid arthritis, filary arthritis and Lyme disease; asthmatic disorders; allergic reactions; and the like. Animals are treated for such disorders by administering an anti-inflammation effective amount of the ether lipid to the animal.

Inflammation is a process of cytological and histological reactions occurring in affected blood vessels, and surrounding tissues, in response to an injury (see, e.g., Stedman's Medical Dictionary (Illustrated) (1982)). Inflammatory responses to such stimuli include local reactions and resulting morphological changes, destruction or removal of injurious materials and activation of repair mechanisms. Thus, inflammation can be part of the process by which animals heal themselves. However, inflammation can also occur in response to abnormal physiological stimuli, and can cause problems in the body. Joints, for example, become inflamed in arthritic conditions such as gout, filary arthritis, rheumatoid arthritis and Lyme disease (see, e.g., Stedman's Medical Dictionary (Illustrated), *supra* at pages 123-124). These states may be characterized by the extravasation of cells, i.e, the egress of cells from the circulation into the inflamed area. Agents which can inhibit such extravasation, or which can otherwise inhibit inflammatory responses to abnormal physiological stimuli, can be used to ameliorate the inflammation.

An "anti-inflammation effective amount" of a liposomal ether lipid is any amount of the ether lipid effective to ameliorate, inhibit or prevent inflammatory responses or reactions in animals afflicted with conditions characterized by abnormal inflammation, i.e., inflammation which is in

response to abnormal physiological stimuli and which is not part of the body's normal repair processes in response to an injury. Anti-inflammation effective amounts of ether lipids are generally from about 0.1 mg of the ether lipid per kg of the body weight of the animal of the animal treated to about 1000 mg of the ether lipid per kg of body weight.

The method of this invention can also comprise administration of an additional biologically active agent to an animal, that is, a biologically active agent in addition to the ether lipid administered. This additional biologically active agent, e.g., an antimicrobial agent, anti-inflammatory agent, antineoplastic agent, therapeutic lipid, or hematopoietic cell growth stimulating agent can be associated, or unassociated, with the liposome. "Association" of a biologically active agent with a liposome means entrapment of the agent in an aqueous compartment or lipid bilayer of the liposome, or association, for example, by means of complex formation, of the agent with the surface of the inner or outer monolayer of the bilayer Preferably, the additional biologically active agent associated with the liposome is an antineoplastic agent, such as an anthracycline antibiotic, antimetabolite or vinca alkaloid. Unassociated bioactive agents can be administered concurrently with, or at different times than, liposome administration. Preferred unassociated biologically active agents are red blood cell growth stimulating agents, e.g., erythropoietin, or interleukin 3 (IL-3) and granulocyte/macrophage colony stimulating factor (GM-CSF).

This invention will be better understood from the following examples. However, those of ordinary skill in the art will readily understand that these examples are merely illustrative of the invention as defined in the claims which follow thereafter.

EXAMPLES

Example 1

Preparation

Chemicals: Ether lipid (EL), egg phosphatidylcholine (EPC), distearoyl phosphatidylcholine (DSPC), dipalmitoyl phosphatidylethanolamine-glutaric acid (DPPE-GA), palmitoyloleoyl phosphatidylethanolamine-glutaric acid (POPE-GA), and dioleoyl phosphatidylethanolamine-glutaric acid (DOPE-GA) were obtained from Avanti Polar Lipids (Birmingham, Alabama). Cholesterol (Chol) was purchased from Sigma Chemical Company U.S.A. Dulbecco's phosphate buffered saline (D-PBS) was purchased from Gibco BRL Life Technologies Inc.(Grand Island, New York, U.S.A). All other reagents were of the highest purity available.

Liposomes: 'Empty' liposomes were prepared by the solvent evaporation method. Liposomes containing EL were prepared by first dissolving EL in chloroform and mixing in EPC/Chol, DOPC/Chol or DSPC/Chol at various mole ratios, with and without 10 mole % of either DPPE-GA, POPE-GA or DOPE-GA in chloroform and methanol (2:1 v/v). The organic solvent was removed under vacuum using a rotary evaporator and the thin dried film was hydrated with a buffer solution of DPBS. The resulting preparations were extruded 10 times through 0.1μm double stacked Nucleopore filters using an extruder device (Lipex Biomembrane Vancouver, BC, Canada). Sizes of liposomes were determined by light scattering a Nicomp Model 370 Submicron Particle Sizer. For all studies, liposomes had a mean diameter between 96-126 nm.

Example 2

Red Blood Cell (RBC) Hemolysis Assay

A 4% suspension of red blood cells (RBCs), 0.5 ml washed three times in PBS, was incubated with various concentrations (serially diluted) of "empty" liposomes, that is, liposomes without ether lipids or other biologically active agents, free (non-liposomal) ether lipid or liposomal ether lipid, prepared as described above. These samples were vortexed on a 37 deg. C. agitator for 20 hours, and were then centrifuged for 10 minutes at 3000 rpm. 0.2 ml of the resulting supernatant was diluted to 1 ml with water, and the percentage hemolysis in the sample was quantitated by spectrophotometric examination at 550 nm. Release of hemoglobin by hypotonic lysis of an equal number of RBC by water was taken as 100% positive control; PBS served as negative control. The ether lipid used in the formulations was 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine (ET-18-O-CH_3).

Results from these studies are presented in Table 1 (see below), the first column indicating the components of the formulation tested for hemolytic activity, the middle column indicating the molar ratios of the respective components, and the third column indicating the lipid concentration (micromolar) at which 5% hemolysis was observed (HI_5).

TABLE 1
Hemolytic Activity

Composition	Molar Ratio	HI_5
Free EL	N/A	5.2
Liposomal Ether Lipid:		
EPC/Chol/EL	4:3:3	20.6
EPC/Chol/DPPE-GA/EL:	1:3:1:5	8.9
	3:3:1:3	14.1
	4:3:1:2	19.0
	5:3:1:1	32.8
EPC/Chol/POPE-GA/EL:	1:3:1:5	13.9
	3:3:1:3	20.1
	4:3:1:2	29.9
	5:3:1:1	31.1
EPC/Chol/DOPE-GA/EL	4:3:1:2	58.7
DOPC/Chol/DOPE-GA/EL	4:3:1:2	106.0
Chol/DPPG/EL	5:3:2	71.8
DSPC/Chol/EL	4:3:3	20.4
DSPC/Chol/DPPE-GA/EL:	1:3:1:5	9.0
	3:3:1:3	10.5
	4:3:1:2	17.9
	5:3:1:1	19.8

Example 3

In Vitro Murine Cancer Cell Growth Inhibition Studies

The specific inhibition of *in vitro* proliferation of various cancer cells by free, liposomal-ET-18-OCH3 or empty (EL free) liposomes was monitored by a Sulforhodamine B (SRB) assay. In brief, 5000 Lewis lung cancer (LLC), P388 or P388-adriamycin resistant (ADR) cells/well were separately plated onto 96 well flat-bottomed microtiter plates in a RPMI-1640 medium supplemented with 10% FBS, and kept at 37°C in a humidified atmosphere of 5% CO_2. After 24 hours, cells were exposed to various concentrations of either empty (EL free) liposomes, DPBS, free ether lipid or liposomal-ether lipid and cultured for another 48 hours at 37°C. Cells treated with various formulations of ET-18-OCH3 were fixed by adding 50 µl of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubated at 4^0C for 1 hour. Plates were rinsed five times with deionized water and left to air dry over night. 100 µl of SRB (0.4% wt/vol in 1% acetic acid) was added and incubated at room temperature for 10-15 minutes. Unbound SRB was removed by washing five times with 1% of acetic acid. Plates were air dried and bound SRB was solubilized with Tris buffer, and optical densities were read at 551 nm and 50% growth inhibition (GI_{50}) was calculated by formula 50 x ((T - T0)/(C-T0)) = 50, where control optical density is (C), test optical density (T) and optical density at time zero is (T0).

Results are presented in Table 2 (see below) as the concentration of ether lipid which achieved fifty percent growth inhibition (GI_{50}), with the first column indicating the composition of the formulation tested, the second column indicating the molar ratios of the respective formulation components and the third, fourth and fifth columns being the GI_{50} data for the LLC, P388 and P388-ADR cultures, respectively. The ether lipid used in the formulations was 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine.

TABLE 2

Growth Inhibition (GI_{50}) of Murine Cancer Cells by Ether Lipid Formulations

Composition	Molar Ratio	LLC	P388	P388-ADR
Free EL	N/A	26.5±5.1	4.2±0.2	6.1±1.6
Liposomal EL:				
EPC/Chol/EL	4:3:3	40.6	----	----
EPC/Chol/POPE-GA/EL:				
	1:3:1:5	44.5±3	5.6±0.4	10.8±0.4
	3:3:1:3	63.2±4	5.2±0.1	9.8±0.4
	4:3:1:2	73.6±0.5	5.3±0.2	9.6±0.4
	5:3:1:1	>75	5.9±0.5	12.3±0.4
EPC/Chol/DPPE-GA/EL:				
	1:3:1:5	28.9	----	----
	3:3:1:3	30.6	----	----
	4:3:1:2	>75	5.1±1.3	8.2±4.3
	5:3:1:1	>60	----	----
EPC/Chol/DOPE-GA/EL:				
	4:3:1:2	71.9	7.5	13.3
DOPC/Chol/DOPE-GA/EL				
	4:3:1:2	67.6±3.3	5.7±0.2	11.5±0.4
Chol/DPPG/EL	5:3:2	>75	11.2	24.3
DSPC/Chol/EL	4:3:3	36.6	----	----
DSPC/Chol/DPPE-GA/EL:				
	3:3:1:3	25.1	----	----

Example 4

In Vitro Human Cancer Cell Growth Inhibition Studies

Human A549 lung cancer, MCF7 and MCF7/ADR cells were plated (5000 cells per well), and after 24 hours incubation, were exposed to various concentrations of ether lipid, either free or liposomal, for 72 hours. Growth inhibition was determined using the SRB assay. Incubation with control liposomes, not containing ether lipid, did not cause growth inhibition at lipid concentrations up to 60 μM. Results are presented in Table 3 (see below) as the concentration of ether lipid which achieved fifty percent growth inhibition (GI_{50}), with the first column indicating the composition of the formulation tested, the second column indicating the molar ratio of the formulation's constituents, and the third, fourth and fifth columns indicating the results for A549, MCF7 and MCF7/ADR cultures, respectively. The ether lipid used in the formulations was the 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine.

TABLE 3

Growth Inhibition (GI_{50}) of Human Cancer Cells by Ether Lipid Formulations

Composition	Molar Ratio	A549	MCF7	MCF7/ADR
Free EL	N/A	6.7±2.5	20.2±7.7	28.4±4.5
Liposomal EL				
EPC:Chol:POPE-GA	1:3:1:5	9.9±0.1	19.1±1.8	34.9±1.8
	3:3:1:3	11.8±0.2	29.1±1.7	41±0.4
	4:3:1:2	10.8±0.4	31.2±2.2	45.1±0.7
	5:3:1:1	18.3±0.1	>66	>75
EPC:Chol:DPPE-GA:EL:				
	4:3:1:2	9.2±3.4	31.6±0.5	55.7±0.1
EPC:Chol:DOPE-GA:EL:				
	4:3:1:2	15.8	36	>68.6
DOPC:Chol:DOPE-GA:EL:				
	4:3:1:2	14.4±0.4	29.4±14.6	>63.4
Chol:DPPG:EL	5:3:2	33.5	>75	>75

Example 5

In Vivo Toxicity Studies

Groups of female C57/BL6 mice (5 /group, weight 20-22 gm) were injected with various doses of free (12.5-200 mg/kg) and liposomal-ether lipid (12.5-200 mg/kg) in PBS via the tail vein. Ethanol was also injected in a separate group of mice. The mice were observed for immediate, acute toxicity: results are presented in Table 4 (see below).

TABLE 4

IN VIVO TOXICITY

Formulation	**Ether Lipid Dose** (mg/kg)	**# Dead Animals**
Ethanol Control	—	0/5
Free EL:		
	200	5/5
	100	5/5
	50	4/5
	25	1/5
	12.5	0/5
	6.25	0/5
Liposomal EL:		
EPC/Chol/GA/EL (5/3/1/1)	100	0/5
EPC/Chol/GA/EL (4/3/1/2)	130	0/5

Example 6

In Vivo Efficacy Studies

Lewis Lung cancer cells(LLC) were obtained from the ATCC and maintained in RPMI medium. Four groups (10/group) of female C57/BL6 mice (body weight 18-22 gm) were injected with $2x10^5$ cells of LLC suspended in 0.2 ml sterile PBS via the tail vein on day 0. On days 1,3,5,7,9 following tumor injection, the first and second groups received either 25 mg/kg free EL or liposomal-EL via the tail vein. The third and fourth groups were administered either the same amount of lipid dosed as EL free liposomes o with 0.2 ml PBS. The *in vivo* anti-metastatic activity of free and liposomal-EL was evaluated on the basis of number of tumor nodules present in the lungs of experimental groups of mice. Results are presented in Figures 1 and 2.

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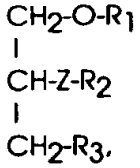
What is claimed is:

1. A liposome having a lipid bilayer comprising an ether lipid and a headgroup-derivatized lipid, wherein the ether lipid has the chemical formula:



wherein R_1 is Y_1Y_2, Y_2 is CH_3 or CO_2H and Y_1 is $(CH_2)_{n1}(CH=CH)_{n2}(CH_2)_{n3}(CH=CH)_{n4}(CH_2)_{n5}(CH=CH)_{n6}(CH_2)_{n7}(CH=CH)_{n8}(CH_2)_{n9}$;

wherein the sum of n1 +2n2 +n3 +2n4 +n5 +2n6 + n7 +2n8 +n9 is equal to an integer of from 3 to 23, n1 is zero or an integer of from 3 to 23, n3 is zero or an integer of from 1 to 21, n5 is zero or an integer of from 1 to 18, n7 is zero or an integer of from zero to 15, n9 is zero or an integer of from 1 to 12, and each of n2, n4, n6 and 8 is independently zero or 1;

wherein Z is O or S;

wherein R_2 is Y_1Y_2 or $(C(X_1)_{n10}(X_2)_{n11})_{n12}CX_3X_4X_5$;

wherein each of X_1, X_2, X_3, X_4, and X_5 is independently H or F, n10 is zero, 1 or 2, n11 is zero, 1, or 2, n12 is zero or an integer of from 1 to 23, and the sum of n10 + n11 is equal to 2 when n12 is not zero;

wherein R_3 has the formula R_5-$P(O)_2$-O-R_6, R_5 is O, S or NH, R_6 is $CH_2CH_2N(CH_3)_3$, $CH_2CH_2NH_2$, $CH_2CH(OH)CH_2OH$, or $CH_2CH_2NHC(O)R_7$ and wherein R_7 is Y_2CH_3 or Y_2CO_2H.

2. The liposome of claim 1, wherein the liposome has a diameter of less than about 200 nm.

3. The liposome of claim 2, wherein the liposome has a diameter of from greater than about 50 nm to less than about 200 nm.

4 The liposome of claim 1, wherein Y_2 is CH_3.

5 The liposome of claim 4, wherein R_1 is $(CH_2)_{n1}CH_3$.

6. The liposome of claim 5, wherein R_1 is $(CH_2)_{17}CH_3$.

7. The liposome of claim 1, wherein Z is O.

8. The liposome of claim 1, wherein R_2 is $(C(X_1)_{n10}(X_2)_{n11})_{n12}CX_3X_4X_5$.

9. The liposome of claim 8, wherein n12 is zero.

10. The liposome of claim 9, wherein R_2 is CH_3.

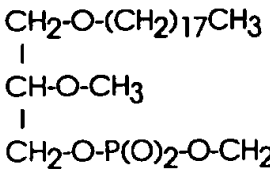
11. The liposome of claim 8, wherein n12 is one.

12. The liposome of claim 11, wherein R_2 is CH_2CH_3, CH_2CF_3 or CF_2CF_3.

13. The liposome of claim 1, wherein R_3 is $O\text{-}P(O)_2\text{-}O\text{-}R_6$.

14. The liposome of claim 13, wherein R_3 is $\text{-}O\text{-}P(O)_2\text{-}O\text{-}CH_2CH_2N(CH_3)_3$.

15. The liposome of claim 1, wherein the ether lipid is



16. The liposome of claim 1, wherein the headgroup-derivatized lipid is a phosphatidylethanolamine-dicarboxylic acid.

17. The liposome of claim 16, wherein the phosphatidylethanolamine is dipalmitoyl phosphatidylethanolamine, palmitoyloleoyl phosphatidylethanolamine or dioleoyl phosphatidylethanolamine.

18. The liposome of claim 17, wherein the phosphatidylethanolamine is dioleoyl phosphatidylethanolamine.

19. The liposome of claim 16, wherein the dicarboxylic acid is glutaric, sebacic, succinic or tartaric acid.

20. The liposome of claim 19, wherein the dicarboxylic acid is glutaric acid.

21. The liposome of claim 16, wherein the phosphatidylethanolamine-dicarboxylic acid is dioleoyl phosphatidylethanolamine-glutaric acid.

22. The liposome of claim 1, wherein the headgroup-derivatized lipid is a circulation-enhancing lipid.

23. The liposome of claim 1, wherein the bilayer comprises a sterol.

24. The liposome of claim 23, wherein the sterol is cholesterol.

25. The liposome of claim 1, wherein the bilayer comprises a neutral lipid.

26. The liposome of claim 25, wherein the neutral lipid is a phosphatidylcholine.

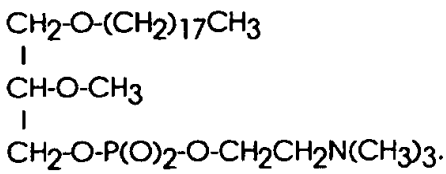
27. The liposome of claim 26, wherein the phosphatidylcholine is egg phosphatidylcholine, palmitoyloleoyl phosphatidylcholine or dioleoyl phosphatidylcholine.

28. The liposome of claim 27, wherein the phosphatidylcholine is dioleoyl phosphatidylcholine.

29. The liposome of claim 1, comprising a circulation-enhancing lipid.

30. The liposome of claim 1, further comprising a sterol and a neutral lipid.

31. The liposome of claim 30, wherein the ether lipid is:



32. The liposome of claim 30, wherein the headgroup-derivatized is dioleoyl phosphatidylethanolamine-glutaric acid.

33. The liposome of claim 30, wherein the sterol is cholesterol.

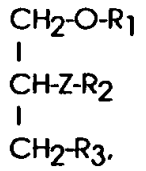
34. The liposome of claim 30, wherein the neutral lipid is dioleoyl phosphatidylcholine.

35. The liposome of claim 30, wherein the bilayer comprises about 20 mole percent of the ether lipid, about 10 mole percent dioleoyl phosphatidylethanolamine-glutaric acid, about 30 mole percent cholesterol and about 40 mole percent dioleoyl phosphatidylcholine.

36. The liposome of claim 1, comprising an additional bioactive agent.

37. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the liposome of claim 1.

38. A method of administering an ether lipid to an animal which comprises administering to the animal a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a liposome having a lipid bilayer comprising the ether lipid and a headgroup-derivatized lipid, wherein the ether lipid has the chemical formula:



wherein R_1 is Y_1Y_2, Y_2 is CH_3 or CO_2H and Y_1 is $(CH_2)_{n1}(CH=CH)_{n2}(CH_2)_{n3}(CH=CH)_{n4}(CH_2)_{n5}(CH=CH)_{n6}(CH_2)_{n7}(CH=CH)_{n8}(CH_2)_{n9}$;

wherein the sum of n1 +2n2 +n3 +2n4 +n5 +2n6 + n7 +2n8 +n9 is equal to an integer of from 3 to 23, n1 is zero or an integer of from 3 to 23, n3 is zero or an integer of from 1 to 21, n5 is zero or an integer of from 1 to 18, n7 is zero or an integer of from zero to 15, n9 is zero or an integer of from 1 to 12, and each of n2, n4, n6 and 8 is independently zero or 1;

wherein Z is O or S;

wherein R_2 is Y_1Y_2 or $(C(X_1)_{n10}(X_2)_{n11})_{n12}CX_3X_4X_5$;

wherein each of X_1, X_2, X_3, X_4, and X_5 is independently H or F, n10 is zero, 1 or 2, n11 is zero, 1, or 2, n12 is zero or an integer of from 1 to 23, and the sum of n10 + n11 is equal to 2 when n12 is not zero;

and wherein R_3 has the formula R_5-$P(O)_2$-O-R_6, R_5 is O, S or NH, R_6 is $CH_2CH_2N(CH_3)_3$, $CH_2CH_2NH_2$, $CH_2CH(OH)CH_2OH$, or $CH_2CH_2NHC(O)R_7$ and R_7 is Y_2CH_3 or Y_2CO_2H.

39. The method of claim 38, wherein the animal is a human.

40. The method of claim 38, wherein the animal is afflicted with a cancer and wherein an anticancer effective amount of the ether lipid is administered.

41. The method of claim 40, wherein the cancer is a lung, brain, colon, ovarian or breast cancer.

42. The method of claim 40, wherein the anticancer effective amount of the ether lipid is from about 0.1 mg of the ether lipid per kg of the body weight of the animal to which the pharmaceutical composition is administered to about 1000 mg of the ether lipid per kg of body weight.

43. The method of claim 38, wherein the animal is afflicted with an inflammatory disorder and an anti-inflammation effective amount of the ether lipid is administered.

44. The method of claim 43, wherein the inflammatory disorder is an arthritic condition, asthmatic disorder or allergic reaction

45. The method of claim 43, wherein the anti-inflammation effective amount of the ether lipid is from about 0.1 mg of the ether lipid

per kg of the body weight of the animal to which the pharmaceutical composition is administered to about 1000 mg of the ether lipid per kg of body weight.

46. The method of claim 38, comprising administering an additional biologically active agent to the animal.

47. The method of claim 46, wherein the additional biologically active agent is an antineoplastic agent, antimicrobial agent, anti-inflammatory agent, therapeutic lipid or hematopoietic cell growth stimulating agent.

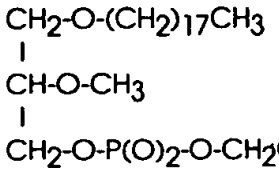
48. The method of claim 38, wherein the liposome has a diameter of from greater than about 50 nm to less than about 200 nm.

49. The method of claim 38, wherein R_1 is $(CH_2)_{17}CH_3$.

50. The method of claim 38, wherein R_2 is CH_3.

51. The method of claim 38, wherein R_3 is $-O-P(O)_2-O-CH_2CH_2N(CH_3)_3$.

52. The liposome of claim 38, wherein the ether lipid is

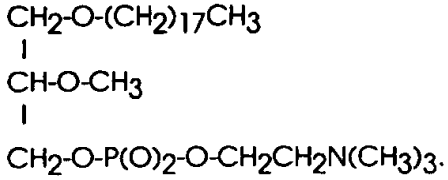


53. The method of claim 38, wherein the headgroup-derivatized lipid is a phosphatidylethanolamine-dicarboxylic acid.

54. The method of claim 53, wherein the phosphatidylethanolamine-dicarboxylic acid is dioleoyl phosphatidylethanolamine-glutaric acid.

55. The method of claim 38, wherein the bilayer further comprises a sterol and a neutral lipid.

56. The method of claim 55, wherein the headgroup-derivatized lipid is dioleoyl phosphatidylethanolamine-glutaric acid, the sterol is cholesterol, the neutral lipid is dioleoyl phosphatidylcholine and the ether lipid is:



57. The method of claim 56, wherein the bilayer comprises about 20 mole percent of the ether lipid, about 10 mole percent dioleoyl phosphatidylethanolamine-glutaric acid, about 30 mole percent cholesterol and about 40 mole percent dioleoyl phosphatidylcholine.

58. The method of claim 38, wherein the liposome comprises a circulation-enhancing lipid.

Fig. 1

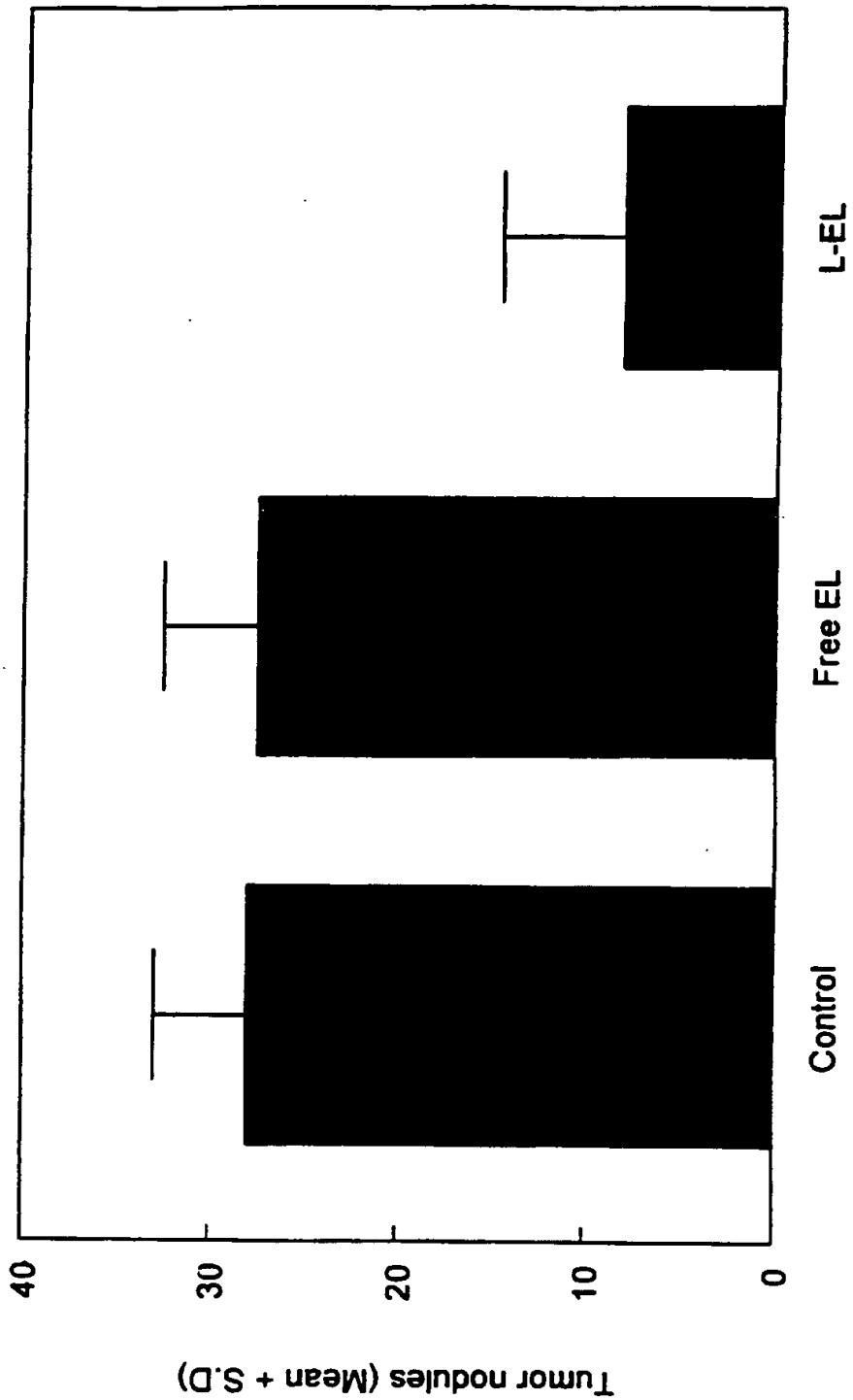
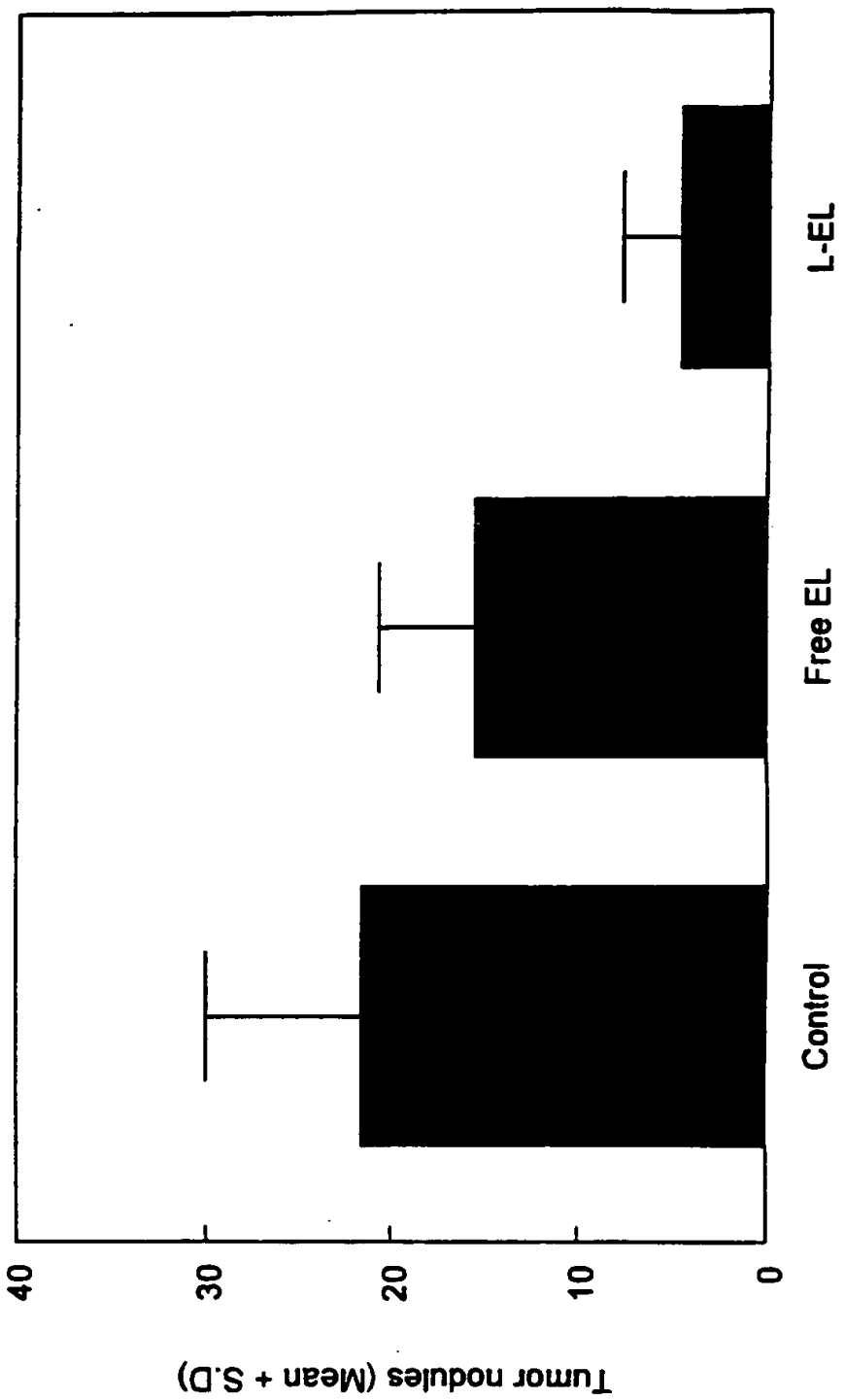


Fig. 2



INTERNATIONAL SEARCH REPORT

Ir tional Application No
PCT/US 95/12721

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/127 A61K31/685

According to International Patent Classification (IPC) or :› both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE,A,41 32 345 (MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN EV) 1 April 1993	1-11, 13-15, 22-24, 29,31, 33, 36-52,58
Y	see page 1, line 1 - page 5, line 30	16-21, 25-28, 30,32, 34,35, 53-55,57
	see page 7, line 25 - page 8, line 6	
	see claims 1-14	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 1996	07.02.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Benz, K

INTERNATIONAL SEARCH REPORT

In ional Application No
PCT/US 95/12721

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	DE,C,44 08 011 (MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN) 2 November 1995 see column 3 - column 4; example 4 see claims 1,3,10	1-11,13, 15, 22-24, 29,31, 33, 36-52,58
Y	EP,A,0 213 523 (THE BOARD OF REGENTS THE UNIVERSITY OF TEXAS SYSTEM) 11 March 1987 see claim 1	16-21, 25-28, 30,32, 34,35, 53-55,57
P,Y	WO,A,94 27580 (THE LIPOSOME COMPANY, INC.) 8 December 1994 cited in the application see claims 1-15,18-22	16-21, 25-28, 30,32, 34,35, 53-55,57

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12721

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 38-58 are directed to method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

In ional Application No
PCT/US 95/12721

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE-A-4132345	01-04-93	NONE		
DE-C-4408011	02-11-95	NONE		
EP-A-213523	11-03-87	AU-B-	591162	30-11-89
		AU-B-	6155686	26-02-87
		CA-A-	1273575	04-09-90
		JP-A-	62096431	02-05-87
		US-A-	4983397	08-01-91
WO-A-9427580	08-12-94	AU-B-	6956894	20-12-94
		CA-A-	2160118	08-12-94
		NO-A-	954691	20-11-95